---o---

A QUANTITATIVE METHOD FOR MICROINJECTING CONTROLLED QUANTITIES OF AQUEOUS SOLUTIONS INTO LIVING CELLS[1]

SAMUEL A. CORSON,

Department of Physiology, University of Oklahoma School of Medicine, Oklahoma City

During the course of some experiments dealing with the permeability of living cells to electrolytes it was found desirable to compare the effects produced by the immersion of the cells into certain solutions with those produced by the microinjection of the same solutions by means of a Chambers micromanipulator. To do this it was necessary to devise some quantitative method for introducing equal volumes of solutions into different cells. This was accomplished by enclosing the desired quantity of solution in the micropipette between two layers of oil, utilizing the fact that most oils are immiscible with protoplasm or with any aqueous solutions in which the cells may be placed.

Before the injection, an oil having a specific gravity significantly higher than that of the solution to be injected is first drawn into the micropipette. A mixture of brominated olive oil and mineral oil, giving a specific gravity of 1.061, was found to be most satisfactory. The solution

[1] Parts of this work were done at the Laboratories of Physiology and Biophysics, U. of Texas, Austin, the Marine Biological Laboratory, Woods Hole, Mass., and the Physiology Laboratories of the U. of Pennsylvania.

[1] Most of this work was done at the laboratory of Prof. Robert Chambers of New York University, to whom I am greatly indebted for valuable suggestions and continuous encouragement.

to be injected is then drawn into the pipette. The oil permits better control of the intake and of the ejection of the solution. Finally a very small quantity of an oil of a low specific gravity and of a relatively low surface tension is drawn into the pipette tip. Olive oil plus a trace of oleic acid was suitable for this purpose. The low surface tension of the light oil in the tip of the pipette permits better control of the injection because such an oil can be readily ejected by the application of gentle pressure on the plunger of the syringe. Differences in the specific gravity of the two oils make it possible to enclose any desired quantity of the experimental solution between the two oil layers. When the injection is made, the minute drop of light oil enters the cell first. This is followed by the experimental solution which can be made to exude at a controlled rate. The heavy oil remains in the micropipette and can be used again for subsequent injections.

The droplet of the injected light oil serves three purposes. (1) Its presence in the cell indicates the actual injection of the solution into the cell. This is particularly important when colorless solutions are used. (2) It prevents the contamination of the solution in the micropipette with that of the hanging drop in which the cells are placed. It also prevents leakage of the fluid from the microtip into the surrounding medium. (3) By using oil droplets of different sizes or colors it is possible to identify cells injected at different times or with different solutions.

The quantity of fluid to be injected is measured by placing the pipette in a plane perpendicular to that of the long axis of a microscope fitted with a calibrated ocular micrometer. All of the solution thus taken in is then injected into a living cell. The pipettes are calibrated by immersion in cedar oil. Since the refractive index of the latter approaches that of the glass, it is possible to measure directly the inner diameter of the pipette and calculate the volume. The results can also be checked by ejecting aqueous solutions from the pipette into an oil of the same specific gravity and then measuring the diameters of the aqueous droplets. It was thus possible to measure quantities of fluid equal to 10_{-8} ml. This is of the order of magnitude of the volume of an ameba nucleus.